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BENZO(A)PYRENE ACCUMULATION AND DEPURATION IN THE SOFT-SHELL CLAM (*MYA ARENARIA*)

by

Eugene Jackim
Laure Wilson
Environmental Research Laboratory
Environmental Protection Agency
Narragansett, Rhode Island 02882

ABSTRACT

Three hundred clams were placed in sediment in a continuous flow seawater tank between March and June. The tank was dosed with ^{14}C labeled benzo(a)pyrene in no. 2 fuel oil daily. Animals were removed weekly and assayed for accumulation of the radioactive material. On the sixth, thirty-third and sixty-first day of exposure animals were removed from the dosing tank, placed in sand substrate with flowing seawater for depuration and again monitored weekly for remaining radioactivity. It was found that above a large variability and fluctuation there was a gradual trend toward increased accumulation over a 90 day period. Depuration is moderately rapid and resembles a first order decay curve. Biological half-life ranged between 5.5 and 9 days. The depuration rate appeared to be somewhat slower upon prolonged accumulation. This is compared to PNA depuration rates from field animals.

INTRODUCTION

Because of the carcinogenic properties of benzo(a)-pyrene and other polynuclear aromatic hydrocarbons, several investigators have recently studied the accumulation and release of these compounds in various shellfish. Bories *et al.* (1976) studied the uptake and depuration of hydrocarbons in mussels (*Mytilus edulis*) from an oil spill and found that benzo(a)pyrene concentrations are down to trace levels after 11 days in clean water, giving an estimated biological half-life of about 2 days. Dunn and Stich (1976) found that mussels removed from a creosote contaminated area, released benzpyrene exponentially for 6 weeks with a biological half-life of about 16 days. Neff and Anderson (1975) found that clams (*Rangia cuneata*) exposed to benzo(a)pyrene for 24 hours retained the compound with a half-life of under 5 days. Clark and Finley (1975) found similar depuration rates with other hydrocarbons in *Mytilus edulis*. On the other hand, Blumer *et al.* (1970) using the oyster *C. virginica*, and more recently Boehm and Quin (1977) studying the hard shell clam, *Mercenaria mercenaria* conclude that after chronic accumulation, depuration is very slow and about 70% of the hydrocarbon remain after 120 days of depuration. In general it appears that even with the same species and the same hydrocarbon there is considerable variation in depuration rates and in the levels of the long lasting residual compartments. Stegeman and Teal (1973), Fassato and Conzonier (1976) and DiSalvo *et al.* (1975) speculate on the existence of biological hydrocarbon pools with different turnover rates in shellfish. The hypothesis suggested is that upon prolonged exposure, slow turnover compartments accumulate more of the hydrocarbon and thus retain it longer than with short-term exposure.

The current study was designed to test this hypothesis by determining if polycyclic aromatic hydrocarbon (PAH) depuration takes longer with prolonged accumulation than with only a short exposure period. Benzo(a)-pyrene labeled with ^{14}C is used in this study as a representative PAH.

MATERIALS AND METHODS

Mya arenaria weighing between 1.7 and 35.5 g were collected from relatively clean clam beds near Pt. Judith, R.I. On March 14th the animals were placed randomly in a 1 m diameter circular fiberglass tank containing 12 cm of beach sand substrate and covered with 20 cm of seawater. The flow rate was 4 ℓ per minute. Dosing consisted of daily addition of 1 μCi(0.1 ml) ^{14}C labeled benzo(a)pyrene (51mCi/mmol), representing a 10 μCi/ml solution in no. 2 fuel oil. At the time of addition, the seawater is turned off for six hours and a recirculating pump turned on. The recirculating water tended to mix the surface slick with the water column. The water drain was designed to receive water from below the surface to prolong contact between the surface slick and water even after resuming the seawater flow. The tank drain emptied through an acrylic fiber filter box which effectively removed all radioactivity and oil (Hyland *et al.*, 1977). The tank water was periodically monitored for radioactivity which never exceeded 7 (DPM) per ml. Water temperature ranged from 6°C at the beginning of the experiment to 20°C at the end.

At weekly intervals 10 clams were removed from the tank at random with regard to size and processed for ^{14}C counting. On the sixth, thirty-third and sixty-first day of dosing 40 clams were removed from the tank and placed in a depuration trough containing sand substrate and flowing seawater at 3 ℓ per minute. Ten clams from each of these depuration groups were removed weekly for counting.

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The counting procedure consisted of shucking the clams and individually weighing the entire contents excluding the shell. The flesh was partially digested by adding 5 mg of a 1:1 mixture of tryspin and pepsin and stirring overnight with a magnetic stirrer at room temperature. To the digest was added 1 to 5 ml of "Soluene 300*" depending on sample size to complete the digestion. Ten parts of "Aquasol" scintillation fluid was added to one part of digest for scintillation counting. If quenching was too high (below 0.5) due to excessive color, the sample was split and more "Aquasol" was added. Carbon-14 activity was measured directly in DPM with a Packard 3380 liquid scintillation counter using the absolute activity analyzer.

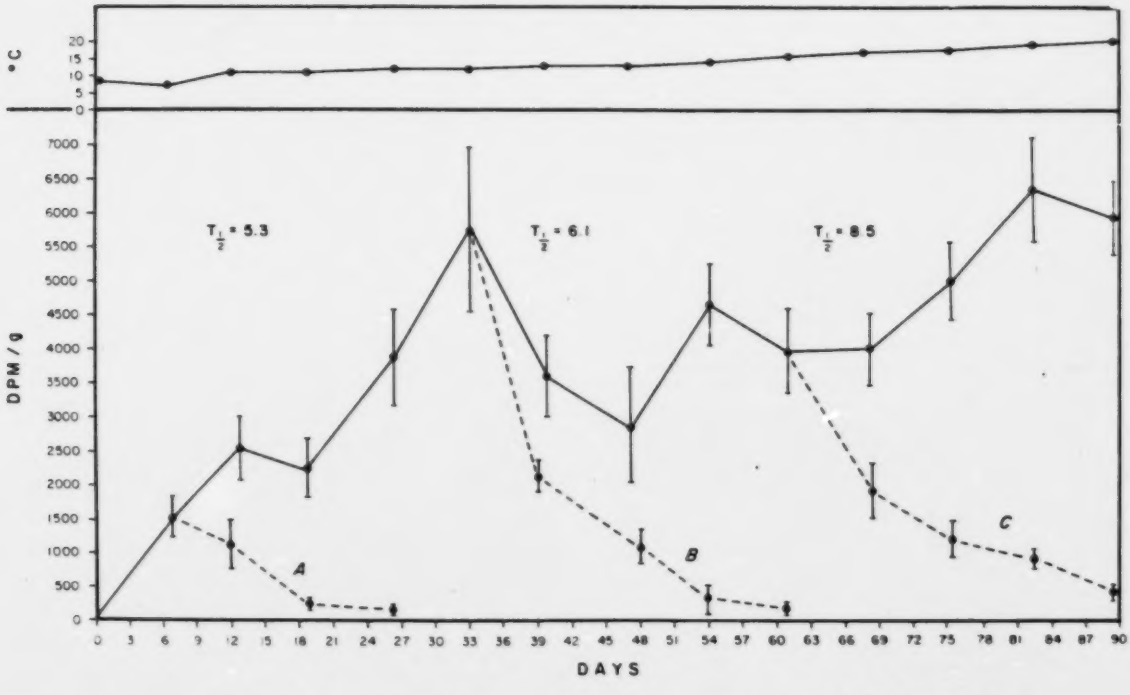
Mya arenaria were collected in March from Quonset Point, R.I. which is chronically polluted with industrial wastes. 5 clams were frozen and stored for analysis at a later date. The remaining clams were placed in clean sand substrate in laboratory tanks with clean flowing seawater of ambient temperature (6-20°C). Five clams were removed after 1, 2, 3 and 5.5 weeks of depuration, shucked and frozen. The samples were processed for hydrocarbon analysis by a modification of MacLeods 1976 procedure and analyzed by gas chromatography-mass spectroscopy techniques for individual compounds.

RESULTS AND DISCUSSION

Benzo(a)pyrene accumulation expressed as DPM/g tissue fluctuated widely with an overall tendency to increase over a 90 day exposure period (Fig. 1). Such wide variability also reported for B(a)P accumulation by other workers (Neff & Anderson, 1975), probably results from a variety of factors including changes in turbidity and phytoplankton content. Depuration generally approximated a first order decay curve with some deviation. The biological half-life ($t_{1/2}$) for the three depuration curves (A,B,C) was 5.3, 6.1 and 8.5 days respectively, see (Figs. 1 and 2). Assuming that much of the first week's depuration is due to clearing of the gut and the remaining depuration is cleansing of the tissue, we re-examined the apparent biological half-life for each depuration group starting with the second week in clean water. The apparent $t_{1/2}$ changes slightly and becomes 4.6, 6.1 and 11.2 days for each of the depuration groups respectively. Collectively, these results tend to support the hypothesis that depuration is appreciably slower after prolonged exposure than after an acute exposure.

It must be noted that although the depuration rate slowed significantly with time, a completely stable residual concentration was not seen in this experiment. Longer exposure might produce such stable residues.

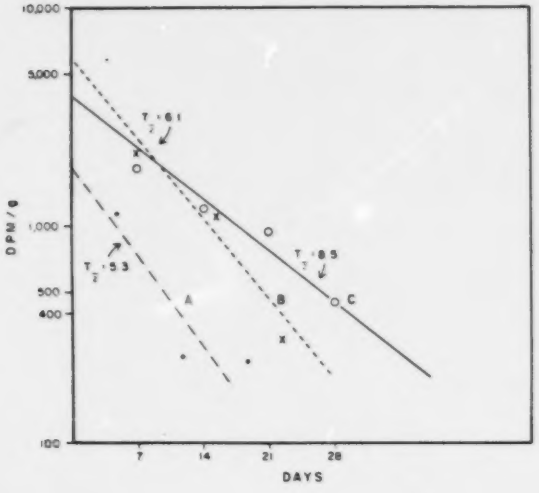
FIGURE 1 Accumulation and depuration of ^{14}C labeled benzo(a)pyrene in *Mya arenaria*[a]



[a]The solid line represents the accumulation rate and dashed lines A, B and C represent depuration starting at 6, 32, and 60 days respectively.

The lack of any consistent sharp breaks in the depuration curves favor against the existence of a small number of biological compartments with distinct retention times, with possible exception of the first week's depuration which would reflect stomach contents. More frequent sampling however, might uncover such compartments.

FIGURE 2 Semi-logarithmic plots of the three depuration curves shown in Figure 1



It must be noted that another variable during the course of the experiment was a seasonal increase in temperature (Fig. 1). This however, should increase total metabolic activity along with depuration rate. Thus, it is unlikely that the slower depuration concurring with the increased exposure time is a result of a seasonal temperature increase. Depuration rates found in *Mya arenaria* in this study, ($t_{1/2}$ between 5.3 and 8.5 days) are in the same range as those found in other shellfish as mentioned previously.

TABLE 1 Radioactive B(a)P Activity in Clam Parts after 1 Week Depuration[a]

	Viscera	Siphon	Mantle	Aductor Muscle	Gills	Juice
$\bar{X}$	23,158	8,349	22,818	10,905	26,086	363
SE	9,066	1,727	15,463	2,331	6,389	28

[a]DPM/g tissue (wet weight)

An analysis of B(a)P in clam parts after a 1 week depuration (shown in Table 1) indicates that concentration is relatively equal throughout the soft tissue with exception of the syphon and abductor muscle which possess about half the concentration of other organs. In a clam without prior depuration the gut appears to contain the largest amount of B(a)P radioactivity as shown by whole body autoradiography (Fig. 3). Polycyclic aromatic hydrocarbon localization in *Mya* is relatively unimportant from the human health viewpoint, since all soft tissue is generally consumed as food.

FIGURE 3 Autoradiograph of ^{14}C benzo(a)pyrene exposed *Mya arenaria*[a]

[a]The clam was exposed to 1 μc of the isotope in 2 ℓ of seawater for 1 week. The film was contact exposed for 30 days. The light areas indicate areas of high radioactivity concentration.

The chronically exposed field clams from a contaminated area near Quonset Point depurated very much like the laboratory model. The initial benzo(a)pyrene concentration was 35 μg/Kg and dropped to 0.76 μg/Kg at the end of 5.5 weeks. The depuration followed a typical exponential decay which displayed a biological half-life of exactly 7 days. Other PAH's behaved similarly (Table 2).

TABLE 2 Depuration of PNA Hydrocarbons [μg/Kg (Wet Weight)][a]

	Weeks Depurated				% Remaining
	1	2	3	5½	
Phenanthrene	194	36	0.9	2.7	1.4
Fluoranthene	653	300	23.0	4.6	0.7
Pyrene	619	233	19.0	1.8	0.3
Benzofluorene	49	27	3.5	trace	0
Chrysene	175	101	19.0	8.4	4.8
Benzo(a)pyrene	30	16	5.2	0.8	2.6

[a]Several identifiable PAH's found in *Mya arenaria* from Quonset Point, Rhode Island and depurated over a period of 5.5 weeks. Each number represents a sample from five pooled clams.

CONCLUSIONS

The laboratory depuration experiment as well as the chronic naturally exposed clams indicate that PAH's are rapidly and nearly completely depurated (92-99%). The length of exposure has only a slight influence on the depuration rate. Laboratory models with short or long exposures appear to simulate field situations adequately for this species. The chronically exposed field clams also show that all of the PAH's tested behave quite similarly with respect to extent of depuration and rate of depuration.

Blumer *et al.* (1970) and Boehm and Quinn (1977) have reported finding very slow and only partial depuration in shellfish. Blumer's earlier work using soxlet extraction and gas chromotographic analysis have been clouded by interfering compounds. It is questionable whether oysters have a longer hydrocarbon retention time than other marine bivalves on the basis of other studies–Stegeman and Teal (1973) and Anderson (1975). Boehm and Quinn also did not resolve the hydrocarbons into individual compounds which, although unlikely, could leave room for interference by unidentified compounds. It is also possible that *M. mercenaria* handle hydrocarbons differently from other shellfish.

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